Research Institute for Fragrance Materials, Inc.
2 University Plaza, Suite 406,
Hackensack, New Jersey 07601-6209 USA
Phone: 201-488-5527 FAX: 201-488-5594

VIA Mail and e-mail

August 9, 2002

Dr. Scott A. Masten
Office of Chemical Nomination and Selection
NIEHS/NTP
P.O. Box 12233, MD A3-07
Research Triangle Park, North Carolina 27709

RE: Ethanone, 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthalenyl) (Iso E Super) [54464-57-2]

Dear Dr. Masten:

This is in response to the June 12, 2002 announcement in the *Federal Register* for information on substances nominated to the NTP for toxicological studies. Ethanone, 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthalenyl or OTNE is a fragrance ingredient that has been evaluated by The Research Institute for Fragrance Materials, Inc. (RIFM).

Attached is a fragrance material review on this material. All studies are on file at RIFM. The International Fragrance Association (IFRA) is currently conducting another use level survey; I will keep you apprised of any updates. In addition, if any additional data become available, I will notify you.

If there is anything more I can do, please do not hesitate to contact me.

Sincerely,

Anne Marie Api

Anne Marie Api, Ph.D.
Scientific Director

Enc/1

FRAGRANCE MATERIAL REVIEW ON 1-(1,2,3,4,5,6,7,8-OCTAHYDRO-2,3,8,8-TETRAMETHYL-2-NAPHTHALENYL)ETHANONE (OTNE)

Research Institute for Fragrance Materials, Inc. Two University Plaza, Suite 406, Hackensack, N.J. 07601 U.S.A.

Telephone: 201-488-5527
Fax: 201-488-5594

SUMMARY

In 1999, a complete literature search was conducted on 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone (OTNE). On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

The acute oral and dermal toxicity of OTNE is very low. Oral and dermal LD_{50}'s in rats were greater than 5 g/kg.

Although slight to moderate skin irritation was observed in animals, no reactions were observed in humans at concentrations up to 2.5%. A 12.5% solution gave slight irritation following a cumulative exposure.

Although slight eye irritation was observed in animals, it may be attributed to the vehicle. OTNE is not considered to be an eye irritant in humans under the recommended current conditions of use as a fragrance ingredient.

There is no evidence of skin sensitization in humans.

Based on the available evidence, it can be concluded that OTNE does not have potential for photoirritation or photoallergy.

Data from an *in vitro* study showed a percutaneous absorption of 16.5%.

A four-week oral (gavage) toxicity study in rats showed that the no-observed-adverse-effect-level (NOAEL) and no-effect-level (NOEL) were 150 mg/kg/day and 15 mg/kg/day, respectively.

Based on data from an oral (gavage) developmental study in rats, the maternal and developmental NOAEL was 240 mg/kg/day.

OTNE is not genotoxic.

Based on the above considerations, the margin of safety for the exposure of humans to OTNE in cosmetic products may be calculated as 1744.

1. Identification

1.1 Synonyms: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-napthyl)ethan-1-one; Ethanone, 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-napthalenyl)-; Bosivelone; Iso E super; Isocyclemone E; OTNE
1.2 CAS Registry Number: 54464-57-2
1.3 EINECS Number: 259-174-3
1.4 Formula: $C_{16}H_{26}O$
1.5 Molecular Weight: 234.38

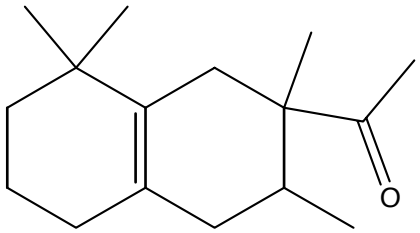

FIGURE 1 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone (OTNE)

2. Physical Properties

2.1 Physical form: Pale straw colored liquid
2.2 Flash point: 232°F
2.3 Log KOW (calculated): 5.23
2.4 Log KOW (measured): 5.6 and 5.7 for 2 isomers
2.5 Specific gravity: 0.96
2.6 Density: 0.96 g/ml at 20°C
2.7 Vapor pressure (calculated): 0.002 mm Hg at 20°C
2.8 Water solubility: 2.0-2.5 ppm
2.9 Specific gravity: 0.96

3. Usage

Table 1: Calculation of the total human skin exposure from the use of multiple cosmetic products containing OTNE

Type of Cosmetic Product	Grams Applied	Applications per day	Retention Factor	Mixture/ Product	Ingredient/ Mixture†	Ingredient mg/kg/day
Body lotion	8.00	0.71	1.000	0.004	20.3	0.0769
Face cream	0.80	2.00	1.000	0.003	20.3	0.0162
Eau de toilette	0.75	1.00	1.000	0.080	20.3	0.203
Fragrance cream	5.00	0.29	1.000	0.040	20.3	0.1962
Antiperspirant	0.50	1.00	1.000	0.010	20.3	0.0169
Shampoo	8.00	1.00	0.010	0.005	20.3	0.0014
Bath products	17.00	0.29	0.001	0.020	20.3	0.0003
Shower gel	5.00	1.07	0.010	0.012	20.3	0.0022
Toilet soap	0.80	6.00	0.010	0.015	20.3	0.0024
Hair spray	5.00	2.00	0.010	0.005	20.3	0.0017
Total						**0.52**

†Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products

OTNE is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its worldwide use is in the region of >1000 metric tons per annum.

The 97.5%ile use level in formulae for use in cosmetics in general has been reported to be 20.3% (IFRA, 1998), which would result in a maximum daily exposure on the skin of 0.52 mg/kg for high end users of these products (See Table 1).

4. Toxicology Data

4.1 Acute Toxicity

Table 2: Summary of acute toxicity data

Route	Species	No. animals/ group	LD_{50}	References
oral	rat	20 (10/sex)	> 5.0 g/kg [> 5000 mg/kg]	RIFM, 1980a
dermal	rat	16 (8/sex)	> 5.0 g/kg [> 5000 mg/kg]	RIFM, 1980b

4.1.1.1 The oral LD_{50} in albino rats exceeded 5.0 g/kg [> 5000 mg/kg] based on no deaths in twenty animals tested at that dose. Ten male and ten female (tacN(SD)fBR) albino rats with initial body weights between 180 and 280 grams received a single oral dose of 5.0 g/kg/body weight [5000 mg/kg/body weight]. Animals were observed for mortality and/or systemic effects at 1, 3, 5 and 24 hours post dose and thereafter, twice daily for 14 days. Gross necropsy was conducted on all animals. There were no systemic effects observed during the study. Gross observations at necropsy were normal for all animals. (RIFM, 1980a).

4.1.1.2 In a dose-range finding study for a 4 week subchronic study, male and female albino (Crl:CD® BR VAF PLUS™) Charles River Sprague-Dawley rats (3/sex/dose) with initial body weights between 124-135 grams were dosed orally for 7 consecutive days with 0.5, 0.75 or 1.0 g/kg/day [500, 750 or 1000 mg/kg/day] in corn oil at a dosage volume of 5.0 ml/kg/day. Control animals received corn oil alone at a dose volume of 5.0 ml/kg/day. Animals were observed for mortality and/or systemic effects, 4 times on day 1 and 3 times per day subsequently. Bodyweights were measured on days 1, 4 and 8 and food consumption was recorded daily. Increased salivation in rats receiving the mid- and high-dose was the only clinical sign observed. No deaths occurred during the study and all animals were sacrificed on day 8. Necropsy was conducted on all animals. Macroscopic observations were recorded and liver, kidneys and spleen were weighed. OTNE had no effect on body weights, body weight changes, or food consumption. No macroscopic abnormalities were observed in any animals; spleen and kidney weights were comparable to controls. Slightly increased liver weights were observed in male and female rats at all dose levels but was only considered to be treatment-related at the high-dose level. Based on these

findings, the high dose for the 4 week subchronic study was set at 1.0 g/kg/day [1000 mg/kg/day] (RIFM, 1995).

4.1.2 Dermal Studies

4.1.2.1 The dermal LD_{50} in Sprague-Dawley CD strain albino rats exceeded 5.0 g/kg/body weight [> 5000 mg/kg/body weight] based on no deaths in sixteen animals tested at that dose. Eight male and eight female rats weighing between 180 and 280 grams were treated on clipped, dorsal skin with a single open application of 5.0 g/kg/body weight [5000 mg/kg/body weight] OTNE. Animals were observed for mortality and/or systemic effects at 1, 3, 5 and 24 hours post dose and twice daily, thereafter, for 14 days. Gross necropsy was conducted on all animals. There were no systemic effects observed during the study. Gross observations at necropsy were normal for all animals (RIFM, 1980b)

References: Research Institute for Fragrance Materials, Inc. (1980a) Acute oral toxicity study with OTNE in rats. Unpublished report from IFF Incorporated, 30 August. Report number 19987.

Research Institute for Fragrance Materials, Inc. (1995) Seven day oral toxicity study of OTNE in the rat. Unpublished report from IFF Incorporated, 8 February. Report number 31710.

Research Institute for Fragrance Materials, Inc. (1980b) Acute dermal toxicity of OTNE in rats. Unpublished report from IFF Incorporated, 30 August. Report number 19988.

4.2 Skin Irritation

4.2.1 Human Studies

Table 3: Summary of Skin Irritation Studies in Humans with OTNE

Method	Dose	Exposure time (hours)	Results	Reference
Induction phase (HRIPT)	2.5% in alcohol SDA 39C	Nine (24 hr) exposures	No reactions (0/36)	RIFM, 1973a
Induction phase (HRIPT)	2.5% in alcohol SDA 39C	Nine (24 hr) exposures	No reactions (0/44)	RIFM, 1977a
Induction phase (HRIPT)	2.5% in alcohol SDA 39C	Nine (24 hr) exposures	No reactions (0/45)	RIFM, 1978a
Induction phase (HRIPT)	12.5% in alcohol SDA 39C	Nine (24 hr) exposures	very slight to slight irritation was observed in 8/51 subjects	RIFM, 1979

4.2.1.1 As part of a human repeated insult patch test, irritation was evaluated during the induction phase for a 2.5% solution of OTNE in alcohol SDA 39C. A 0.5 ml dose of the test sample was applied to semi-occlusive patches, which were then applied to the upper arm of each subject. These patches were removed 24 hours after application. A total of nine applications (3 times a week) were made over a three week period. No irritation was observed in 9 male and 27 female volunteers (RIFM, 1973a).

4.2.1.2 Irritation was evaluated during the induction phase in a second human repeated insult patch test which was conducted on 44 volunteers (8 males and 36 females). A 0.4 ml dose of a 2.5% solution of OTNE in alcohol SDA 39C was applied to semi-occlusive patches, which were then applied to the upper arm of each subject. These patches were removed 24 hours after application. A total of nine applications (3 times a week) were made over a three week period. No irritation was observed (RIFM, 1977a)

4.2.1.3 Irritation was evaluated during the induction phase in a human repeated insult patch test conducted on 45 volunteers with 2.5% OTNE in alcohol SDA 39C. Subjects received a total of nine 24 hour occluded induction applications

over a three week period. No irritation was observed (RIFM, 1978a).

4.2.1.4 As part of a human repeated insult patch test in 51 volunteers (8 males and 43 females), irritation was evaluated during the induction phase for a 12.5% solution in alcohol SDA 39C. A 0.2 ml aliquot of the test sample was applied to the webril pad of an occluded Parke-Davis Readi-Bandage which was then applied to the back of each subject for 24 hours. A total of nine applications (3 times a week) were made over a three week period. Very slight to slight irritation was observed in 8 volunteers (RIFM, 1979)

4.2.1.5 Irritation was evaluated during the induction phase of an associated photosensitization study. One male and 27 female volunteers were tested with a 12.5% solution of OTNE in alcohol SDA 39C. A 0.1 ml aliquot of the test material was applied to filter paper patches which were then applied to a $1cm^2$ area on the back for 24 hours under semi-occlusion. After an interval of 48 hours, the test material was patched again at the same site. A total of six applications, (2 times a week) were made over a three week period. Reactions were read just prior to the next patch application. No irritant effects were observed (RIFM, 1980c)

4.2.2 Animal Studies

Table 4: Summary of Skin Irritation Studies in Animals with OTNE

Method	Dose	Exposure time (hours)	Results	Reference
Primary irritation screen for a guinea pig sensitization study	0.62 % in alcohol SDA 39C	4 hour occluded application	very slight to slight erythema in 2/4 guinea pigs	RIFM, 1973b
Primary irritation screen for a guinea pig sensitization study	1.25 % in alcohol SDA 39C	4 hour occluded application	very slight to slight erythema in 2/4 guinea pigs	RIFM, 1973b
Primary irritation screen for a guinea pig sensitization study	2.5 % in alcohol SDA 39C	4 hour occluded application	moderate erythema and edema in 3/4 guinea pigs	RIFM, 1973b
Primary irritation screen for a guinea pig sensitization study	5.0 % in alcohol SDA 39C	4 hour occluded application	no reactions (0/4)	RIFM, 1973b
Primary irritation screen for a guinea pig sensitization study	10 % in alcohol SDA 39C	4 hour occluded application	moderate erythema and edema in 3/4 guinea pigs	RIFM, 1973b
Primary irritation screen for a guinea pig sensitization study	20 % in alcohol SDA 39C	4 hour occluded application	moderate erythema and edema in 3/4 guinea pigs	RIFM, 1973b
Primary irritation study in rabbits	2.5% in alcohol SDA 39C	24 hour occluded application	no reactions (0/3)	RIFM, 1973c
Primary irritation study in rabbits	2.5% in alcohol SDA 39C	24 hour occluded application	no reactions (0/3)	RIFM, 1977b
Primary irritation study in rabbits	2.5% in alcohol SDA 39C	24 hour occluded application	no reactions (0/3)	RIFM, 1978b

4.2.2.1 A primary irritation screen was conducted in guinea pigs (4/dose) prior to a sensitization study with OTNE at dose levels of 0.62%, 1.25%, 2.5%, 5.0%, 10% and 20% in alcohol SDA 39C. A 0.5 ml dose of the test material was applied to a 3/4 x 1 inch webril pad of a Duke Elastopatch bandage which was then applied to clipped skin on the back for 4 hours under occlusion. Reactions were read 24 hours after

patch removal. No irritation was observed at 5.0%; very slight to slight erythema was observed in 2 animals at both 0.62% and 1.25%; moderate erythema and edema were observed in 3 animals at dose levels of 2.5%, 10% and 20%. Based on these findings a dose level of 2.5% was selected for the sensitization study (RIFM, 1973b)

4.2.2.2 OTNE was evaluated for irritation in 3 healthy, albino rabbits. A 0.5 ml dose of a 2.5% solution of the test sample in alcohol SDA 39C was applied to intact skin and another 0.5 ml dose was applied to abraded skin; both were applied under occlusion for 24 hours. Skin reactions were evaluated by the Draize scoring method at 24 and 72 hours after application. No irritation was observed. The primary irritation score was 0. The material was not considered to be a primary skin irritant (RIFM, 1973c).

4.2.2.3 OTNE was evaluated for irritation in 3 albino rabbits. A 0.5 ml dose of a 2.5% solution of the test sample in alcohol SDA 39C was applied to intact skin and another 0.5 ml dose was applied to abraded skin; both were applied under occlusion for 24 hours. Skin reactions were evaluated by the Draize scoring method at 24 and 72 hours after application. No irritation was observed (RIFM, 1977b). Using the same protocol as above, a 2.5% solution of OTNE in alcohol SDA 39 C was again evaluated for irritation in 3 additional albino rabbits. No irritation was observed (RIFM, 1978b).

References:

Research Institute for Fragrance Materials, Inc. (1973a) Repeated insult patch test in humans with OTNE. Unpublished report from IFF Incorporated, 26 September. Report number 19978.

Research Institute for Fragrance Materials, Inc. (1977a) Repeated insult patch test in humans with OTNE. Unpublished report from IFF Incorporated, 15 November. Report number 19981.

Research Institute for Fragrance Materials, Inc. (1978a) Repeated insult patch test in humans with OTNE. Unpublished report from IFF Incorporated, 15 August and 29 December. Report number 19984.

Research Institute for Fragrance Materials, Inc. (1979) Evaluation of potential hazards of fragrance materials by dermal contact in humans. Unpublished report from IFF Incorporated, 17 September. Report number 19986.

Research Institute for Fragrance Materials, Inc. (1980c) Photoallergic/phototoxic study in humans with fragrance materials. Unpublished report from IFF Incorporated, 16 December. Report number 19989.

Research Institute for Fragrance Materials, Inc. (1973b) Sensitization study in guinea pigs with OTNE. Unpublished report from IFF Incorporated, 20 December. Report number 19979.

Research Institute for Fragrance Materials, Inc. (1973c) Primary skin irritation study in rabbits with OTNE. Unpublished report from IFF Incorporated, 12 June. Report number 19976.

Research Institute for Fragrance Materials, Inc. (1977b) Primary skin irritation study in rabbits with OTNE. Unpublished report from IFF Incorporated, 27 May. Report number 19982.

Research Institute for Fragrance Materials, Inc. (1978b) Primary skin irritation study in rabbits with OTNE. Unpublished report from IFF Incorporated, 8 March. Report number 19983.

4.3 Mucous Membrane (Eye) Irritation

4.3.1 Animal Studies

Table 5: Summary of Eye Irritation Studies in Animals with OTNE

Concentration	Vehicle	Results	Reference
2.5%	alcohol SDA 39C	mild conjunctivitis in 2/3 rabbits which cleared by day 7 vehicle alone produced mild conjunctival irritation	RIFM, 1973d
2.5%	alcohol SDA 39C	conjunctivitis in 3/3 rabbits with corneal involvement in 2 rabbits; all eyes clear by day 7 vehicle alone produced moderate conjunctival irritation	RIFM, 1977c
2.5%	propylene glycol	no irritation	RIFM, 1978c

4.3.1.1 A Draize rabbit eye irritation test (Draize, 1944) was conducted in 3 healthy, albino rabbits. A 0.1 ml aliquot of a 2.5% solution of OTNE in alcohol SDA 39C was instilled into the right eye of each animal with no further treatment. The untreated left eye of each animal served as a control. Observations were made every 24 hours for 4 days and then again on the 7th day. Scorings were recorded according to the Draize scale for scoring ocular lesions. Mild conjunctival irritation, which cleared by day 7, was observed in 2 rabbits. When the vehicle, alcohol SDA 39C, was tested alone, mild conjunctival irritation was observed in all 3 rabbits with corneal involvement in one rabbit (RIFM, 1973d).

4.3.1.2 A second Draize rabbit eye irritation test (Draize, 1944) was conducted in 3 additional healthy, albino rabbits. A 0.1 ml aliquot of a 2.5% solution of OTNE in alcohol SDA 39C was instilled into the right eye of each animal with no further treatment. The untreated left eye of each animal served as a control. Observations were made every 24 hours for 4 days and then again on the 7th day. Scorings were recorded according to the Draize scale for scoring ocular lesions. Mild conjunctival irritation was observed in all 3 rabbits with corneal involvement in 2 rabbits. All eyes were clear by day 7. When the vehicle, alcohol SDA 39C, was tested alone, moderate conjunctival irritation with corneal involvement was observed in all 3 animals (RIFM, 1977c).

4.3.1.3 A third Draize rabbit eye irritation test (Draize, 1944) was conducted in a further 3 healthy, albino rabbits using a different vehicle. A 0.1 ml aliquot of a 2.5% solution of OTNE in propylene glycol was instilled into the right eye of each animal with no further treatment. The untreated left eye of each animal served as a control. Observations were made every 24 hours for 4 days and then again on the 7th day. Scorings were recorded according to the Draize scale for scoring ocular lesions. No irritation was observed (RIFM, 1978c).

References: Draize J.H., Woodard G. and Calvery H.O. (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmac. exp. Ther.*, **82**, 377-390. [Submitted only for the methodology]

Research Institute for Fragrance Materials, Inc. (1973d) Eye irritation study in rabbits with OTNE. Unpublished report from IFF Incorporated, 3 July. Report number 19977.

Research Institute for Fragrance Materials, Inc. (1977c) Eye irritation study in rabbits with OTNE. Unpublished report from IFF Incorporated, 31 May. Report number 19980.

Research Institute for Fragrance Materials, Inc. (1978c) Eye irritation study in rabbits with OTNE. Unpublished report from IFF Incorporated, 9 March. Report number 19985.

4.4 Skin Sensitization

4.4.1 Human Studies

Table 6: Summary of Human Studies for Skin Sensitization with OTNE

Test Method	Test Concentration	Results	References
HRIPT	2.5% in alcohol SDA 39C	No reactions (0/36)	RIFM, 1973a
HRIPT	2.5% in alcohol SDA 39C	No reactions (0/44)	RIFM, 1977a
HRIPT	2.5% in alcohol SDA 39C	No reactions (0/42)	RIFM, 1978a
HRIPT	12.5% in alcohol SDA 39C	No reactions (0/51)	RIFM, 1979

4.4.1.1 A human repeated insult patch test was conducted with OTNE on 36 volunteers (9 males and 27 females). A 0.5 ml dose of a 2.5% solution of OTNE in alcohol SDA 39C was applied to a 1 inch square Webril swatch affixed to a 1 x 2 inch bandage which was then applied to the upper arm under semi-occlusion. These patches were removed 24 hours after application. After a 24-48 hour rest period, subjects were again patched at the same site. Reactions were read 24-48 hours after patch removal just prior to application of the next patch. A total of nine applications were made over a three week period. Approximately two weeks after the last induction patch, a semi-occluded challenge patch was applied to a site not previously exposed and removed after 24 hours. Reactions to challenge were read at 24 and 72 hours after patch removal. No sensitization reactions were produced (RIFM, 1973a).

4.4.1.2 A second human repeated insult patch test was conducted with 2.5% OTNE on 44 volunteers (8 males and 36 females). A 0.4 ml dose of a 2.5% solution of OTNE in alcohol SDA 39C was applied to a 1 inch square Webril swatch affixed to a 1 x 2 inch bandage which was then applied to the upper arm of each subject under semi-occlusion. These patches were removed 24 hours after application. After a 24-48 hour rest period, subjects were again patched at the same site. Reactions were read 24-48 hours after patch removal just prior to application of the next patch. A total of nine applications were made over a three week period. Approximately two weeks after the last induction patch, a semi-occluded challenge patch was applied to a site not previously exposed and removed after 24 hours. Reactions to challenge were read at 24 and 72 hours after patch removal. No sensitization reactions were produced (RIFM, 1977a).

4.4.1.3 Another human repeated insult patch test was conducted with 2.5% OTNE on an additional 42 volunteers. Subjects received nine 24 hour occluded induction applications over a three week period. An occluded challenge application was made approximately two weeks after the last induction patch. No sensitization reactions were produced (RIFM, 1978a).

4.4.1.4 A human repeated insult patch test was conducted with a 12.5% solution of OTNE in alcohol SDA 39C on 51 volunteers (8 males and 43 females). A 0.2 ml aliquot of the test sample in alcohol SDA 39C was applied to the webril pad of a 1 1/2 inch square occlusive Parke-Davis Readi-Bandage which was then applied to the back of each subject for 24 hours. After a 24-48 hour rest period, subjects were again patched at the same site. Reactions were read at patch removal and again 24-

48 hours after patch removal just prior to application of the next patch. A total of nine applications were made, 3 times a week, over a three week period on a Monday-Wednesday-Friday schedule. Seventeen days after application of the last induction patch, a 24 hour occluded challenge patch was made to the upper arm. Reactions to challenge were read at patch removal and again at 24, 48 and 72 hours after patch removal. No sensitization reactions were produced (RIFM, 1979).

4.4.1.5 As part of a photoallergy test, sensitization was evaluated with 12.5% OTNE in alcohol SDA 39C in one male and 27 female volunteers. A 0.1 ml aliquot of the test material was applied to filter paper patches which were then applied to a $1cm^2$ area on the back for 24 hours under semi-occlusion. After an interval of 48 hours, the test material was patched again at the same site. A total of six applications, (2 times a week) were made over a three week period. Reactions were read just prior to the next patch application. Ten days following the removal of the last induction patch, a 24 hour semi-occluded challenge patch was applied to a new site. Reactions were read after patch removal and again at 24, 48 and 72 hours after patch removal. Sensitization was not observed (RIFM, 1980c).

4.4.1.6 Frosch *et al* (1995) reported on the results of a multicenter study on patch tests with 48 fragrance materials. OTNE, 1% and 5% in petrolatum, was tested in 313 male and female patients (132 males and 181 females). The material was applied to the back for 2 days using Finn chambers® on Scanpor tape®. Reactions were assessed per ICDRG guidelines on days 2 and 3 or on days 2 and 4. One sensitization reaction was observed at each dose level. A Repeated Open Application Test (ROAT) was conducted with 1% OTNE in petrolatum, on the patient who had reacted to 1% OTNE, and produced no reactions.

4.4.1.6 Larsen *et al* (2001) reported on the results of a multicenter study conducted with a fragrance mix and a series of individual fragrance materials, which included 5% OTNE in petrolatum. Patch tests were conducted on 178 patients who previously reacted to either the fragrance mix or an individual material. Patch sites were initially evaluated at 48 or 72 hours with each site being reexamined between 2-5 days following the initial scoring in a majority of the cases. Three sensitization reactions (3/178) were observed to 5% OTNE in petrolatum.

4.4.2 Animal Studies

Table 7: Summary of Animal Studies for Skin Sensitization with OTNE

Test Method	Concentration	Subjects	Results	References
Induction consisted of four 24 hour occluded applications over a 7 day period & an intradermal FCAT injection prior to third induction application. Approximately 10 -12 days later, guinea pigs were challenge by a 24 hour occluded application (method of Maguire, 1973)	2.5% in alcohol SDA 39C	guinea pigs	no reactions (0/10)	RIFM, 1973b

4.4.2.1 Ten guinea pigs were tested in a sensitization study (Maguire, 1973). A 0.2 ml aliquot of a 2.5% solution of OTNE in alcohol SDA 39C was applied to a 2 cm^2 area on the clipped and shaved right quadrant of the back for 24 hours under occlusion. Four induction applications were made to the same site over a 7 day period. Prior to the third induction application, an intradermal injection of 0.1 ml Freund's Complete Adjuvant was made on both sides of the test site. Approximately 10-12 days after the last induction application, the guinea pigs were challenged by an occluded 24 hour patch at a previously untreated site on the upper left quadrant of the back. At the same time, the challenge treatment was applied to 10 naive animals that had not been treated before. Reactions were read at patch removal and again at 24 and 48 hours after patch removal. No sensitization reactions were observed (RIFM, 1973b).

References: Research Institute for Fragrance Materials, Inc. (1973a) Repeated insult patch test in humans with OTNE. Unpublished report from IFF Incorporated, 26 September. Report number 19978.

Research Institute for Fragrance Materials, Inc. (1977) Repeated insult patch test in humans with OTNE. Unpublished report from IFF Incorporated, 15 November. Report number 19981.

Research Institute for Fragrance Materials, Inc. (1978a) Repeated insult patch test in humans with OTNE. Unpublished report from IFF Incorporated, 15 August and 29 December. Report number 19984.

Research Institute for Fragrance Materials, Inc. (1979) Evaluation of potential hazards of fragrance materials by dermal contact. Unpublished report from IFF Incorporated, 17 September. Report number 19986.

Research Institute for Fragrance Materials, Inc. (1980c) Photoallergic/phototoxic study in humans with fragrance materials. Unpublished report from IFF Incorporated, 16 December. Report number 19989.

Frosch P.J., Pilz B., Andersen K.E., Burrows D., Camarasa J.G., Dooms-Goossens A., Ducombs G., Fuchs T., Hannuksela M., Lachapelle J.M., Lahti A., Maibach H.I., Menne T., Rycroft R.J.G., Shaw S., Wahlberg J.E., White I.R. and Wilkinson J.D. (1995) Patch testing with fragrances: Results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Contact Dermatitis*, 33, 333-342.

Larsen W., Nakayama H., Fischer T., Elsner P., Frosch P., Burrows D., Jordan W., Shaw S., Wilkinson J., Marks J., Jr., Sugawara M., Nethercott M. and Nethercott J. (2001). Fragrance contact dermatitis: A worldwide multicenter investigation (Part II). *Contact Dermatitis*, **44**(6), 344-346.

Maguire H.C.,Jr. (1973) The bioassay of contact allergens in the guinea pig. *Journal of the Society of Cosmetic Chemists*, **24**, 151-162. [Submitted only for the methodology]

Research Institute for Fragrance Materials, Inc. (1973b) Sensitization study in guinea pigs with OTNE. Unpublished report from IFF Incorporated, 20 December. Report number 19979.

4.5 Photoirritation

4.5.1 Human Studies

4.5.1.1 Phototoxicity was evaluated during the induction phase of an associated photosensitization study. One male and 27 female volunteers were tested with a 12.5% solution of OTNE in alcohol SDA 39C. A 0.1 ml aliquot of the test material was applied to filter paper patches which were then applied to a 1cm^2 area on the back for 24 hours under semi-occlusion. After patch removal, any excess test material was carefully removed and the test sites were then irradiated with UV for a time period of three times the previously determined MED (UVA + UVB) using a Xenon Arc Solar Simulator (emission spectrum 290-400 nm). After an interval of 48 hours, the test material was patched again at the same site. A total of six applications, (2 times a week) were made over a three week period. Reactions were read 48 hours after irradiation just prior to the next patch application. No phototoxic effects were observed (RIFM, 1980c; Weinberg and Springer, 1981).

4.5.2 Animal Studies

4.5.2.1 A 20 µl aliquot of a 10% solution of OTNE in alcohol SDA 39C was applied to a 5 cm^2 area on the back of 24 male Skh:hairless mice. Thirty minutes later, the treated skin areas were irradiated with UV light for one hour either with a bank of six fluorescent blacklight lamps (Sylvania F20/40T12BL, Phosphor type BL-O) with 800 µw/cm^2 intensity at a distance of 30 centimeters or by a Atlas Xenon lamp, model Rm 65, (wavelength 280-320 nm) with a Schott WG320 filter at a distance of 1 meter. Reactions were read at 4, 24, 48, 72 and 96 hours after exposure. There were no phototoxic effects observed (RIFM, 1980d; Weinberg and Springer, 1981).

4.5.3 Miscellaneous Studies

4.5.3.1 An *in vitro* study on Fleischman's active dry yeast using an agar overlay technique was conducted to determine the phototoxicity of 0.1%, 1.0% and 10% OTNE in methanol. An air dried paper disc impregnated with 40 µl of the test material was added to a microplate along with the yeast suspension, and the plate was irradiated with UV light for 18 hours from Sylvania F15T8 BLB lamps, (emission spectrum, 320-400 nm, peak 370 nm) with a UV surface flux at the plate of 1.5-2 mW/cm^2. Zones of inhibition were measured at 48 hours after inoculation or when the contrast is adequate. No phototoxic effects were observed with 0.1% and 1.0% OTNE in

methanol; positive phototoxic effects were observed with 10% OTNE in methanol (RIFM, 1980e; Weinberg and Springer, 1981).

4.5.3.2 An *in vitro* study using an agar overlay technique with the yeast, *Saccharomyces cerevisiae* was used to evaluate the phototoxic potential of OTNE. An air dried paper disc impregnated with 25 µl of a 5% solution of OTNE in methanol was added to an agar microplate along with a 1% yeast suspension, and the plate was irradiated with UVA from 4 black light tubes (Westinghouse, F40BL, 320-400nm) for 18 hours at a distance of 31 cm, and then incubated for 48 hours. Zones of inhibition were then measured. Phototoxic effects were not observed (Tenenbaum, *et al.,* 1984).

References:

Research Institute for Fragrance Materials, Inc. (1980c) Photoallergic/phototoxic study in humans with fragrance materials. Unpublished report from IFF Incorporated, 16 December. Report number 19989.

Weinberg E.H. and Springer S.T. (1981) The evaluation *in vitro* of fragrance materials for phototoxic activity. *Journal of the Society of Cosmetic Chemists*, **32**, 303-315.

Research Institute for Fragrance Materials, Inc. (1980d) Phototoxicity test in hairless mice with fragrance materials. Unpublished report from IFF Incorporated, 29 February. Report number 19990.

Research Institute for Fragrance Materials, Inc. (1980e) Phototoxicity screen in yeast. Unpublished report from IFF Incorporated, 7 June. Report number 19991.

Weinberg E.H. and Springer S.T. (1981) The evaluation *in vitro* of fragrance materials for phototoxic activity. *Journal of the Society of Cosmetic Chemists*, **32**, 303-315.

Tenenbaum S., DiNardo J., Morris W.E., Wolf B.A. and Schnetzinger R.W. (1984) A quantitative *in vitro* assay for the evaluation of phototoxic potential of topically applied materials. *Cell Biology and Toxicology*, **1**, 1-9.

4.6 Photoallergenicity

4.6.1 Human Studies

4.6.1.1 The photoallergic potential of OTNE in alcohol SDA 39C was evaluated in one male and twenty seven female volunteers. A 0.1 ml aliquot of a 12.5% solution of OTNE in alcohol SDA 39C was applied to filter paper patches which were then applied to a 1cm^2 area on the back for 24 hours under semi-occlusion. Duplicate patch sites were used, one site was exposed to irradiation and one site was covered with a black cloth during irradiation and served as a control. After patch removal, any excess test material was carefully removed and one test site was then irradiated with UV for a time period of three times the previously determined MED (UVA + UVB) using a Xenon Arc Solar Simulator (emission spectrum 290-400 nm). After an interval of 48 hours, the test material was patched again at the same site. A total of six applications, (2 times a week) were made over a three week period. Reactions were read 48 hours after irradiation just prior to the next patch application. Ten days following the sixth light exposure, a 24 hour semi-occluded challenge patch was applied to a new site. After patch removal, the test site was exposed to UV light for 3 minutes from a Xenon Arc Solar Simulator with a WG345 filter (emission spectrum 320-400 nm). Reactions were read at 15 minutes after irradiation and again 24, 48 and 72 hours after irradiation. No photoallergic effects were observed (RIFM, 1980c).

References: Research Institute for Fragrance Materials, Inc. (1980c) Photoallergic/phototoxic study in humans with fragrance materials. Unpublished report from IFF Incorporated, 16 December. Report number 19989.

4.7 Absorption, Distribution and Metabolism

4.7.1 Percutaneous Absorption

4.7.1.1 *In Vitro* Studies in Humans

4.7.1.1.1 An *in vitro* percutaneous absorption study was completed. The study was designed to determine the *in vitro* skin penetration rate and distribution of the radiolabelled material (0.2 mCi OTNE, [6,6-methyl-^{14}C]). The evaporative loss of the labelled test material, under the study conditions was also measured. Horizontal glass diffusion cells were used. The receptor medium was a 50/50 ethanol/water solution that was continuously agitated. The test membrane was human cosmetic reduction skin that was heat separated to yield epidermal membranes comprising both the stratum corneum and the epidermis. The integrity of each membrane was assessed prior to the permeation experiments. The skin surface temperature was maintained at 32˚C. Twelve replicate samples were run, as were 2 untreated control samples. Samples from the receptor fluid were taken at 2, 8, 24, 36, and 48 hours and were analyzed by liquid scintillation. The epidermal membranes were tape stripped 10 times and were grouped, solubilized, and analyzed. The evaporative loss of the test material over a 48 hour period was assessed using PTFE sheets mounted in the diffusion cells. The PTFE sheets were removed at 1, 2, 4, 8, 24, and 48 hours after dosing and washed with solvent. The washings were analyzed by liquid scintillation.

Following 48 hours exposure, 15.3 +/- 1.2% of the applied dose of OTNE (~20 µl/cm^2 of a 1% solution in ethanol) had permeated into the receptor phase. After 24 hours, the receptor phase level of OTNE was 9.0% of applied dose. The total recovery of OTNE from the PTFE surfaces at 48 hours was 57% of the applied dose. The levels of OTNE in the surface wipe and donor chamber wash were 38.5 +/- 2.5 µg/cm^2 and 32.5 +/- 2.8 µg/cm^2, respectively. Overall recovery (surface wipe, tape strips, remaining epidermis, receptor phase and donor chamber) of OTNE was 53.3 +/- 1.4% of the applied dose. The addition of evaporated OTNE to the above recoveries would move values closer to 100% (RIFM, 2000; Isola and Api, 2002). If levels of OTNE in the remaining stratum corneum plus epidermis and permeated OTNE were combined to produce a total absorbed dose value, then 16.51% of the applied dose was absorbed (See Tables 1 and 2).

Table 1: Percent Applied Dose Recovered (Average ± 1 S.E.) after 48 hours

Receptor Phase	Surface Wipe	Skin Strips	Epidermis	Donor Chamber	Recovery
15.3 ± 4.1	19.4 ± 4.2	1.1 ± 0.3	1.21 ± 0.49	16.3 ± 4.8	53.3 ± 4.9

Table 2: Percent of Applied Dose Absorbed

Receptor Phase	Epidermis	Total Absorbed
15.3 ± 4.1	1.21 ± 0.49	16.51

References: Research Institute for Fragrance Materials, Inc. (2000) *In vitro* skin penetration of radiolabelled fragrance materials. RIFM report number 37084.
Isola D.A. and Api A.M. (2002). *In vitro* human skin penetration of seven radiolabelled fragrance materials. *The Toxicologist*, **66**(1-S), 165.

4.8 Subchronic Toxicity

4.8.1 Animal Studies

4.8.1.1. The effects of OTNE when administered daily to male and female rats over a 4 week period followed by a two week recovery period has been reported (RIFM, 1997a). Male and female Sprague-Dawley (Crl:CD® BR VAF PLUS™) rats with initial bodyweights of 67-90 grams were administered OTNE by gavage, once daily for 28 consecutive days, at dosage levels of 15, 150 or 1000 mg/kg/day. OTNE was prepared as a suspension in corn oil at concentrations of 0.3, 3.0 or 20% w/v and was administered at a dosage volume of 5 ml/kg/day. The two lower dose groups consisted of five male and five female rats and the high-dose and control groups consisted of 10 male and 10 female rats. Control animals received 5 ml/kg/day corn oil. Animals were observed three times daily for mortality and/or systemic effects. Water consumption was measured by gravimetric measurement during weeks 3 and 5. Food consumption and bodyweights were recorded weekly. Blood and urine samples were taken prior to sacrifice. All animals were examined macroscopically and specified tissues were prepared for histopathological examination. There were no treatment related mortalities. One female in the high-dose group died on day 44 but this was not considered to be treatment related and

was considered to be due to an anesthetic accident. All rats in the low- and mid-dose groups and five males and five females from the high-dose group were sacrificed following the four week treatment period. The remaining five males and five females were retained for a two week recovery period, following which, they were also sacrificed. During the treatment period yellow/brown staining around the urogenital region and dirty, matted fur was observed in the high-dose group. Also during treatment, bodyweight gains were statistically significantly lower in male rats in the mid- and high-dose groups. Water consumption was higher than control values in the high-dose animals. Food consumption, hematology, biochemistry and urinalysis were all within normal parameters.

At the end of the treatment period, cholesterol levels were higher than controls in males and females in the high-dose group; γ-glutamyltransferase levels were increased for some male and females in the high-dose group and glutamic pyruvic transaminase (GPT) levels were lower in males in the high-dose group. Liver weights were statistically significantly higher than controls in males and females in the high-dose group. Enlarged livers were also noted in males and females in this group; centrilobular hepatocyte enlargement also noted in males and females in the high-dose group was considered to be associated with the increase in organ weights and macroscopically enlarged livers. Eosinophilic inclusions in the cortical tubules in the kidneys of male rats in the mid- and high-dose groups were also noted at the end of treatment. Following the two-week recovery period, higher cholesterol levels for females and lower GPT levels for males were recorded. Eosinophilic inclusions in the cortical tubules in the kidneys of male rats were still noted but to a lesser extent then at the end of the treatment period. No treatment related effects were detected in male rats in the low-dose group or in female rats in the low-and mid-dose groups. Based on the findings in this study, it can be concluded that the NOAEL and NOEL for OTNE when administered orally for at least 4 weeks are 150 mg/kg/day and 15 mg/kg/day, respectively (RIFM, 1997a).

References: Research Institute for Fragrance Materials, Inc. (1997a) Four week oral toxicity study in the rat with two week recovery period. Unpublished report from IFF Incorporated, 13 October. Report number 31713.

4.9 Developmental Toxicity

4.9.1 Animal Studies

4.9.1.1. The developmental toxicity of OTNE was investigated in Crl:CD®(SD) IGS BR VAF/Plus® rats. One hundred (25/group) presumed pregnant female rats were dosed, via gavage, on days 7 through 17 of gestation with the neat material at a volume of 0.1, 0.25 or 0.5 ml/kg respectively. Control animals were treated with reverse osmosis deionized processed water at a dose volume of 0.5 ml/kg. OTNE (97% purity, specific gravity 0.96) was therefore administered at doses of 0.0 (control), 100, 240 and 480 mg/kg/day. Food and water were provided *ad libitum*. Animals were observed twice daily for mortality and morbundity. Clinical observations of test article effects and observations for abortion and premature delivery were conducted before and approximately one hour following dosing and once daily thereafter. Bodyweights were recorded prior to the start of the study and daily during dosage and postdosage periods. Feed consumption was recorded on days 0, 7, 10, 12, 15, 18 and 21. On day 21, all rats were sacrificed by CO_2 asphyxiation, Caesarean sectioned and a gross necropsy conducted on all animals. The uterus of each rat was removed and examined for pregnancy, number and distribution of implantations, fetal mortality and resorptions. Each fetus was removed from the uterus with surviving fetuses being sacrificed by intraperitoneal injection; all fetuses were examined macroscopically for the presence, shape and size of all organs. Fetal bodyweights were recorded. Approximately one half of each litter was examined for soft tissue alterations using a variation of the Wilson's staining technique. The remaining fetuses were eviscerated, cleared, stained and examined for skeletal alterations.

No deaths or premature deliveries were caused by OTNE. Two deaths were observed during the course of the study in the 240 mg/kg/day dose group. One death on day 17 was attributed to handling during intubation. The cause of the second death was undermined, but not considered to be attributable to the test article. One animal in the 240 mg/kg/day dose group was observed to deliver prematurely on day 20. The pups were delivered normally and the delivery was attributed to mistiming of the mating for this animal and was not considered related to the test article. Clinical observations included excessive salivation in all dose groups generally occurring after six to seven doses; red perioral substance occurring in 2, 1 and 3 animals in the 100, 240 and 480 mg/kg/day dose groups

respectively and an increase in urine stained abdominal fur in the highest dose group.

Significant reductions in body weight gain were observed in all dosage groups from gestation days 7 to 10. Body weight gain continued to be reduced significantly only in the 480 mg/kg/day dosage group throughout the dosage period (calculated as gestation days 7 to 18) and from gestation days 7 to 21 an 0 to 21. Maternal body weights and body weight gains were generally comparable from gestation days 10 to 21 for the 0, 100 and 240 mg/kg/day dosage groups. Maternal body weights were significantly reduced on gestation days 16 and 17 in the 480 mg/kg/day dosage group compared to the control group values.

Absolute (g/day) and relative (g/kg/day) feed consumption values were reduced or significantly reduced during the first half of the dosage period (gestation days 7 to 12) in the treated groups compared to the control group. After gestation day 12, absolute and relative feed consumption values were comparable for the control, 100 and 240 mg/kg/day dosage groups. Significant reductions in absolute and relative feed consumption values continued in the 480 mg/kg/day dosage group from gestation days 12 to 15, 7 to 18 (entire dosage period), 0 to 18 and 7 to 21.

Totals of 22, 25, 24 and 25 rats were pregnant. Although not statistically significant, fetal body weights were reduced in the 480 mg/kg/day dosage group. All other Caesarean-sectioning and litter parameters were unaffected by dosages of OTNE as high as 480 mg/kg/day. All fetal gross external, soft tissue or skeletal malformations or variations were considered to be unrelated to ONTE. The number of ossification sites per fetus per litter was unaffected at dosages as high as 480 mg/kg/day

Based on these data, the maternal no-observable-adverse-effect-level (NOAEL) of OTNE is 240 mg/kg/day. The 480 mg/kg/day dosage of OTNE produced persistent clinical observations and reductions in body weights and feed consumption. Dosages of 100 and 240 mg/kg/day caused excess salivation and transient reductions in body weight gains and feed consumption generally only during the first days of the dosage period. The developmental NOAEL is 240 mg/kg/day. The 480 mg/kg/day dosage of OTNE produced a minimal, but not statistically significant reduction in fetal body weights, and no other effects on development (RIFM, 2001).

References: Research Institute for Fragrance Materials, Inc. (2001) Oral (gavage) developmental toxicity study of 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone (OTNE) in rats. RIFM report number 39733, September 10.

4.10 Mutagenicity and Genotoxicity

4.10.1 Bacterial Studies

4.10.1.1 In an Ames test (Ames *et al*., 1975) using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 with and without S9 activation, doses up to 5000 μg/plate OTNE in dimethyl sulfoxide were not mutagenic (RIFM, 1997). Positive controls were 3 μg/plate *N*-Ethyl-*N'*-nitro-*N*-nitrosoguanidine (strain TA100 without activation); 5 μg/plate *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (strain TA1535 without activation); 80 μg/plate 9-aminoacridine (strain TA1537 without activation); 1 μg/plate 2-nitrofluorene (strain TA98 without activation); 2 μg/plate 2-nitrofluorene (strain TA1538 without activation); 0.5 μg/plate 2-aminoanthracene (strains TA1538 and TA98 with activation); 1 μg/plate 2-aminoanthracene (strain TA100 with activation) and 2 μg/plate 2-aminoanthracene (strains TA1535 and TA1537 with activation) (RIFM, 1997b).

4.10.1.2 In a modification of the Ames assay (Ames *et al*., 1975; Green, 1984) using *Escherichia coli* strain WP2 *uvrA trp* with and without S9 activation, doses up to 5000 μg/plate OTNE in dimethyl sulfoxide were not mutagenic. The positive controls were 2 μg/plate *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (without activation) and 10 μg/plate 2-aminoanthracene (with activation) (RIFM, 1997b).

4.10.2 Mammalian Studies

4.10.2.1 A chromosome aberration assay was conducted to assess the ability of OTNE to induce chromosomal aberrations in cultured human lymphocytes. The assay was conducted with and without metabolic activation in cultured human lymphocytes exposed to OTNE, dissolved in dimethyl sulfoxide, using multiple harvest times (18 and 32 hours after initiation of treatment). In a first test, cells were exposed for 18 hours at dose levels of 15, 30 and 50 μg/ml in the absence of a metabolic activation system and at dose levels of 15.6, 62.5 and 125 μg/ml in the presence of an Aroclor-induced rat liver S-9

activation system. In a second test, cells were exposed for 18 hours to OTNE at doses of 7.5, 15 and 30 μg/ml without activation or to doses of 31.25, 62.5 and 125 μg/ml with activation. In the second test, cells were also exposed for 32 hours to 15 μg/ml OTNE without activation and to 75 μg/ml with activation. Ethyl methanesulphonate was used as the positive control in the non-activated tests at final concentrations of 250, 500 and 750 μg/ml and cyclophosphamide was used as the positive control in the S-9 activated tests at final concentrations of 2.5, 5.0, 10 and 15 μg/ml. Colchicine was added 2 hours prior to harvesting, and chromosome samples were prepared by the Giemsa method. To calculate the percentage of chromosome aberration, about 100 metaphase spreads were scored. There were no statistically significant increases in the proportion of aberrant cells in the presence of S-9 mix, at the 18 or 32 hour harvests. In the absence of S-9 mix at the 18 hour harvest, there was a statistically significant increase in the number of aberrant cells at the highest concentration, 30 μg/ml, when gap damage was excluded. However, since this increase (to 4%) lies within the historical control range (0-5.25%), and since there was no dose-response relationship and since this increase was not seen in the first test or at the 32 hour sampling time, it was concluded that this increase was not treatment related. It was further concluded that under the conditions of this test, OTNE showed no evidence of clastogenic activity (RIFM, 1997c).

References:

Ames B.N., McCann J. and Yamasaki E. (1975) Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutation Research*, **31**, 347-363. [Submitted only for the methodology]

Research Institute for Fragrance Materials, Inc. (1997b) Bacterial Mutation Assay with Iso E Super. Unpublished report from IFF Incorporated, 8 October. Report number 31711.

Green M.H.L. (1984) Mutagen testing using *trp*$^+$ reversion in *Escherichia coli* in *Handbook of Mutagenicity Test Procedures*. 2nd edition. Edited by B.J. Kilbey, M. Legator, W. Nichols and C. Ramel. p. 161. Elsevier Science Publishers BV, Amsterdam.

Research Institute for Fragrance Materials, Inc. (1997c) Metaphase chromosome analysis of human lymphocytes cultured *in vitro*.

Unpublished report from IFF Incorporated, 12 August. Report number 31712.

5. Provisional (and conservative) Risk Assessment

5.1 Estimate of Skin Exposure from Cosmetic Products

The estimated skin exposure of humans to OTNE in cosmetic products provided a conservative estimate of potential skin contact of 0.52 mg/kg/day.

5.2 Estimate of Systemic Exposure for Humans

The data from the *in vitro* study showed a percutaneous absorption of approximately 16.5%. Using this figure, the estimate for systemic exposure by humans using cosmetic products is 16.5% of 0.52 mg/kg/day, equal to 0.086 mg/kg/day.

5.3 Safe Exposure Based on Studies in Animals

In the developmental toxicity (oral gavage) study in rats the developmental no-observable-adverse effect level (NOAEL) for OTNE was 240 mg/kg/day.

In the 4-week subchronic oral (gavage) toxicity study in rats the NOAEL and NOEL for OTNE were 150 mg/kg/day and 15 mg/kg/day, respectively.

Based on the above considerations the margin of safety for the exposure of humans to OTNE in cosmetic products may be calculated as:

$$150 \text{ mg/kg/day} \div 0.086 \text{ mg/kg/day} = 1744$$